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## BOX PCT

Commissioner for Patents  
Washington, D.C. 20231

PCT/JP00/04039  
-filed June 21, 2000

Re: Application of Keiichi KAWAI, Norito TAKAMURA and Ryuichi NISHII  
METHOD OF THE ADMINISTRATION OF DRUGS WITH BINDING  
AFFINITY FOR PLASMA PROTEIN AND PREPARATION TO BE USED IN  
THE METHOD  
Assignee: NIHON MEDI-PHYSICS CO., LTD.  
Our Ref: Q67507

Dear Sir:

The following documents and fees are submitted herewith in connection with the above application for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty:

- an executed Declaration and Power of Attorney.
- an English translation of the International Application.
- four (4) sheets of drawings.
- an English translation of Article 34 amendments (annexes to the IPER).
- an executed Assignment and PTO 1595 form.
- Notification Concerning Submission or Transmittal of Priority Document.
- a Preliminary Amendment

It is assumed that copies of the International Application, the International Search Report, and Article 19 amendment as required by § 371(c) will be supplied directly by the International Bureau, but if further copies are needed, the undersigned can easily provide them upon request.

The Government filing fee is calculated as follows:

Total claims	36	-	20	=	16	x	\$18.00	=	\$288.00
Independent claims	2	-	3	=		x	\$84.00	=	\$0.00
Base Fee									\$890.00
Multiple Dependent Claim Fee									\$280.00

<b>TOTAL FILING FEE</b>	<u>\$1458.00</u>
<b>Recordation of Assignment</b>	<u>\$ 40.00</u>
<b>TOTAL FEE</b>	<u>\$1498.00</u>

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Checks for the statutory filing fee of \$1458.00 and Assignment recordation fee of \$40.00 are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. §§ 1.16, 1.17 and 1.492 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from:

<u>Country</u>	<u>Application No</u>	<u>Filing Date</u>
Japan	11-173514	June 21, 1999

Respectfully submitted,

A handwritten signature in blue ink that reads "Sheldon I. Landsman".  
Sheldon I. Landsman  
Registration No. 25,430

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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Keiichi KAWAI, et al.

Appln. No.: NOT YET ASSIGNED

Confirmation No.: NOT YET ASSIGNED

Group Art Unit: NOT YET ASSIGNED

Filed: December 21, 2001

Examiner: NOT YET ASSIGNED

For: METHOD OF THE ADMINISTRATION OF DRUGS WITH BINDING AFFINITY FOR PLASMA PROTEIN AND PREPARATION TO BE USED IN THE METHOD

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

**IN THE CLAIMS:**

Please add the following new claims:

14. Method of the administration of drugs with binding affinity for plasma protein, which is characterized in that, in the administration of a first drug with binding affinity for plasma protein, a single or plural second drug with binding affinity for the same plasma protein for which the first drug has binding affinity, is administered simultaneously with the first drug or before or after the administration of the first drug to thereby regulate the binding of the first drug to the plasma protein.

15. The method of the administration of drugs with binding affinity for plasma protein according to Claim 14, wherein the second drug has binding affinity to the same binding sites on plasma protein to which the first drug has binding affinity.

16. The method of the administration of drug with binding affinity for plasma protein according to Claim 14, wherein the first drug is a radiodiagnostic drug for in vivo use or a radiotherapeutic drug for in vivo use.

17. The method of the administration of drugs with binding affinity for plasma protein according to Claim 15, wherein the first drug is a radiodiagnostic drug for in vivo use or the radiotherapeutic drug for in vivo use.

18. The method of the administration of drugs with binding affinity for plasma protein according to Claim 16 or 17, wherein the radiodiagnostic drug for in vivo use or the radiotherapeutic drug for in vivo use is radiolabeled with one nuclide selected from the group consisting of 11-carbon (<sup>11</sup>C), 15-oxygen (<sup>15</sup>O), 18-fluorine (<sup>18</sup>F), 32-phosphorus (<sup>32</sup>P), 59-iron (<sup>59</sup>Fe), 67-copper (<sup>67</sup>Cu), 67-gallium (<sup>67</sup>Ga), 81m-krypton (<sup>81</sup>Kr), 81-rubidium (<sup>81</sup>Rb), 89-strontium (<sup>89</sup>Sr), 90-yttrium (<sup>90</sup>Y), 99m-technetium (<sup>99m</sup>Tc), 111-indium (<sup>111</sup>In), 123-iodine (<sup>123</sup>I), 125-iodine (<sup>125</sup>I), 131-iodine (<sup>131</sup>I), 133-xenon (<sup>133</sup>Xe), 117m-tin (<sup>117m</sup>Tn), 153-samarium (<sup>153</sup>Sm), 186-rhenium (<sup>186</sup>Re), 188-rhenium (<sup>188</sup>Re), 201-thallium (<sup>201</sup>Tl), 212-bismuth (<sup>212</sup>Bi), 213-bismuth (<sup>213</sup>Bi) and 211-astatine (<sup>211</sup>At).

19. The method of the administration of drugs with binding affinity for plasma protein according to Claim 16 or 17, wherein the first drug has one group labeled with nuclide and the group is selected from the group consisting of bisaminothiol or its derivatives, monaminomonooamidobisthiol or its derivatives, bisamidobisthiol or its derivatives, mercaptoacetylglycylglycylglycine or its derivatives, hexamethylpropyleneamineoxime or its

derivatives, ethylenebis [bis(2-ethoxyethyl) phosphine] or its derivatives, 2,3-dimercaptosuccinic acid or its derivatives, ethylenecysteine dimer derivatives, methoxyisobutylisonitrile derivatives, polyamine derivatives, pyridoxylideneaminate derivatives, methylene diphosphonate, hydroxymethylene diphosphonate derivatives,  $\beta$ -methyl- $\omega$ -phenylpentadecanoic acid or its derivatives, N-isopropylamphetamine, hippuric acid and benzylguanidine and tropane derivatives.

20. The method of the administration of drugs with binding affinity for plasma protein according to any one of claims 14 to 17, wherein the single or plural second drug is selected from the group consisting of bucolome, cefazolin, etoposide, phenylbutazone, aspirine, salicylic acid, cefatrixone, sulfamethizole, valproic acid, nabumetone, 6-methoxy-6-naphthyl acetic acid, ibuprofen, probenecid, dansyl-L-asparagine, verapamil and disopyramide.

21. A pharmaceutical preparation for regulating binding affinity of a first drug for plasma protein, which comprises a first drug with binding affinity for plasma protein and a single or plural second drug with binding affinity for the same plasma protein, for which the first drug has binding affinity.

22. The pharmaceutical preparation according to Claim 21, wherein each of the first drug and the second drug is separately filled in a container, and prepared as a kit form for supply.

23. The pharmaceutical preparation according to Claim 21, wherein the second drug has binding affinity to the same binding sites on the plasma protein, to which the first drug has binding affinity.

24. The pharmaceutical preparation according to Claim 22, wherein the second drug has binding affinity to the same binding sites on the plasma protein, to which the first drug has binding affinity.

25. The pharmaceutical preparation according to any one of Claims 21 to 24, wherein the first drug is a radiodiagnostic drug for in vivo use or a radiotherapeutic drug for in vivo use.
26. The pharmaceutical preparation according to Claim 25, wherein the radiodiagnostic drug for in vivo use or the radiotherapeutic drug for in vivo use is radiolabeled with one nuclide selected from the group consisting of 11-carbon (<sup>11</sup>C), 15-oxygen (<sup>15</sup>O), 18-fluorine (<sup>18</sup>F), 32-phosphorus (<sup>32</sup>P), 59-iron (<sup>59</sup>Fe), 67-copper (<sup>67</sup>Cu), 67-gallium (<sup>67</sup>Ga), 81m-krypton (<sup>81m</sup>Kr), 81-rubidium (<sup>81</sup>Rb), 89-strontium (<sup>89</sup>Sr), 90-yttrium (<sup>90</sup>Y), 99m-technetium (<sup>99m</sup>Tc), 111-indium (<sup>111</sup>In), 123-iodine (<sup>123</sup>I), 125-iodine (<sup>125</sup>I), 131-iodine (<sup>131</sup>I), 133-xenon (<sup>133</sup>Xe), 117m-tin (<sup>117m</sup>Sn), 153-samarium (<sup>153</sup>Sm), 186-rhenium (<sup>186</sup>Re), 188-rhenium (<sup>188</sup>Re), 201-thallium (<sup>201</sup>Tl), 212-bismuth (<sup>212</sup>Bi), 213-bismuth (<sup>213</sup>Bi) and 211-astatine (<sup>211</sup>At).
27. The pharmaceutical preparation according to Claim 25, wherein the first drug has one group labeled with nuclide and the group is selected from the group consisting of bisaminothiol or its derivatives, monoaminomonooamidobisthiol or its derivatives, bisamidobisthiol or its derivatives, mercaptoacetylglycylglycylglycine or its derivatives, hexamethylpropyleneamineoxime or its derivatives, ethylenebis [bis (2-ethoxyethyl) phosphine] or its derivatives, 2,3-dimercaptosuccinic acid or its derivatives, ethylenecysteine dimer derivatives, methoxyisobutylisonitrile derivatives, polyamine derivatives, pyridoxylydeneamine derivatives, methylene diphosphonate, hydroxymethylene diphosphonate derivatives,  $\beta$ -methyl- $\omega$ -phenylpentadecanoic acid or its derivatives, N-isopropylamphetamine, hippuric acid, benzylguanidine and tropane derivatives.
28. The pharmaceutical preparation according to any one of Claims 21 to 24, wherein the single or plural second drug is selected from the group consisting of bucolome, cefazolin, etoposide, phenylbutazone, aspirine, salicylic acid, ceftriaxone, sulfamethizole, valproic acid,

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nabumetone, 6-methoxy-2-naphthylacetic acid, ibuprofen, probenecid, dansyl-L-asparagine, verapamil and disopyramide.

29. The pharmaceutical preparation according to Claim 25, wherein the single or plural second drug is selected from the group consisting of bucolome, cefazoline, etoposide, phenylbutazone, aspirine, salicylic acid, ceftriaxone, sulfamethizole, valproic acid, nabumetone, 6-methoxy-2-naphthylacetic acid, ibuprofen, probenecid, dansyl-L-asparagine, verapamil and disopyramide.

#### **REMARKS**

Entry and consideration of this Amendment is respectfully requested.

Respectfully submitted,

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Date: December 21, 2001

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 1-13 are canceled.

Claims 14-29 are added as new claims.

#### **DESCRIPTION**

METHOD OF THE ADMINISTRATION OF DRUGS WITH  
BINDING AFFINITY FOR PLASMA PROTEIN AND  
PREPARATION TO BE USED IN THE METHOD

## 5 TECHNICAL FIELD

The present invention relates to a method of  
the administration of drugs with binding affinity for  
plasma protein and drugs regulating the effective  
ingredient dose of drugs with binding affinity for  
plasma protein; and a pharmaceutical preparation whereby  
the effective ingredient dose of drugs with binding  
affinity for plasma protein is regulated.

## BACKGROUND ART

Generally, drugs administered for the purpose of medical treatment or diagnosis once go through the systemic blood circulation, and then take the process of absorption, distribution, metabolism, excretion and the like. In the process of absorption and distribution, the drug moves along on flow of the blood, while it transfers to each spaces of intravascular, intersticel and intracellular by diffusion and transportation of a free drug being in the state of unbound form with proteins, and finally the drug arrives at the active region of target. When movement of the drug reaches a steady state, then the free drug concentration in each space become uniform, thus the whole pattern of the

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concentration of the drug is determined by the binding level with proteins. Hence, in accordance with the property, a drug *in vivo*, may partially exists in the form of reversible binding state with biopolymers such  
5 as plasma proteins. Generally, drugs permeable through capillary wall or cell membrane are free drugs, therefore, the transfer of such free drugs being unbound with plasma proteins to the active region of target may be greatly influenced by the binding level with plasma  
10 proteins.

For example, mercaptoacetylglycylglycylglycine labeled with  $^{99m}$ -technetium ( $^{99m}$ Tc-MAG<sub>3</sub>) is widely used in renal scintigraphy, especially the renal plasma flow can be effectively exhibited by its efficient renal extraction and renal tubular secretion. It is known that about 90% of  $^{99m}$ Tc-MAG<sub>3</sub> binds to plasma protein in an ordinary clinical dose (Bubeck B. et al., J. Nucl. Med., 31, 1285-1295, 1990). If the binding of  $^{99m}$ Tc-MAG<sub>3</sub> with plasma protein is inhibited by drugs having high  
15 binding affinity to the same binding site on protein with  $^{99m}$ Tc-MAG<sub>3</sub>, then more clear renal imaging can be obtained in the earlier stage after the administration, thus it may be thought that the dose of radioactivity to the patient can be reduced at the same time.  
20

On the contrary, if the binding of drugs with plasma protein is increased, then the concentration of the free drugs in the blood can be kept in lower level for long period, therefore, it may be possible to  
25

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achieve continuous appearance of pharmacological effects.

However, at the present stage, little is known as the research work for improving therapeutic effect or diagnostic effect of the drugs by regulating the concentrations of the free drugs, using the binding affinity of the second drug with plasma proteins.

#### DISCLOSURE OF THE INVENTION

In consideration of the above-mentioned problems, an object of the present invention is to provide a suitable method of the administration of drugs by regulating the binding affinity of the drug for plasma proteins, and at the same time, to provide a pharmaceutical preparation whereby the binding affinity of the drug for plasma protein can be regulated.

According to the present invention, the suitable administration of the drugs can be achieved by regulating the binding affinity of the drug for plasma protein, and at the same time, a pharmaceutical preparation for such administration can be provided.

The present invention relates to a method of administration of drugs with binding affinity for plasma protein, characterized by regulating the binding affinity of the first drug for plasma protein, when administering the first drug with the binding affinity for plasma protein, the second drugs, which have the binding affinity for the same plasma protein, for which

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the first drug has binding affinity, is administered simultaneously with the first drug or before or after the administration of the first drug.

Particularly, in the case of regulating the binding affinity of the first drug for plasma protein, it is preferable that the first drug and the second drugs bind to the same binding sites on the plasma protein. Further, the second drug may be administered before, after or simultaneously with the administration of the first drug, and such administration timing of the second drug can be suitably selected in connection with the timing when the free drug concentration of the first drug reaches to the level so as to obtain an adequate effect. Additionally, a single drug may be used as the second drug, or plural drugs may be used as the second drugs when synergistic effect can be expected.

In case of administering the first drug and the second drug simultaneously, the pharmaceutical preparations comprising the first drug and the second drug may be supplied. Further, the first drug and the second drug may be filled in a container separately, and may be supplied as a kit form. In case of such a kit form with separate containers, they may be administered simultaneously by mixing it together when used, or each one of the first drug and the second drug can be administered in different times separately or by different route. Furthermore, both of or either one of these first and second drugs may be commercially

available pharmaceuticals.

When the first drug is radiodiagnostic agent for in vivo use or radiotherapeutic drug for in vivo use, the radioactive nuclides can be selected from the group comprising 11-carbon (<sup>11</sup>C), 15-oxygen (<sup>15</sup>O), 18-fluorine, (<sup>18</sup>F), 32-phosphorus (<sup>32</sup>P), 59-iron (<sup>59</sup>Fe), 67-copper (<sup>67</sup>Cu), 67-gallium (<sup>67</sup>Ga), 81m-krypton (<sup>81m</sup>Kr), 81-rubidium (<sup>81</sup>Rb), 89-strontium (<sup>89</sup>Sr), 90-yttrium (<sup>90</sup>Y), 99m-technetium (<sup>99m</sup>Tc), 111-indium (<sup>111</sup>In), 123-iodine (<sup>123</sup>I), 125-iodine (<sup>125</sup>I), 131-iodine (<sup>131</sup>I), 133-xenon (<sup>133</sup>Xe), 117m-tin (<sup>117m</sup>Sn), 153-samarium (<sup>153</sup>Sm), 186-rhenium (<sup>186</sup>Re), 188-rhenium (<sup>188</sup>Re), 201-thallium (<sup>201</sup>Tl), 212-bismuth (<sup>212</sup>Bi), 213-bismuth (<sup>213</sup>Bi) and 211-astatine (<sup>211</sup>At).

In this case, compounds, such as chelating group or receptor ligand, of the first drug labeled with the above-mentioned nuclides can be selected from bisaminothiol or its derivatives, monoaminomonoamidobisthiol or its derivatives, bisamido-bisthiol or its derivatives, mercaptoacetylglycylglycylglycine or its derivatives, hexamethylpropylene-amineoxime or its derivatives, ethylenebis[bis(2-ethoxyethyl)phosphine] (tetrofosmin) or its derivatives, 2,3-dimercaptosuccinic acid or its derivatives, ethylenecysteine dimer derivatives, methoxyisobutylisonitrile derivatives, polyamine derivatives, pyridoxylidineamine derivatives, methylene diphosphonate, hydroxymethylene diphosphonate

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derivatives,  $\beta$ -methyl- $\omega$ -phenylpentadecanoic acid or its derivatives, N-isopropyl-amphetamine, hippuric acid, benzylguanidine, tropane derivatives and the like.

The second drug may be selected from, for example, bucolome, cefazolin, etoposide, phenylbutazone, aspirine, salicylic acid, cefatriaxone, sulfamethizole, valproic acid, nabumetone, 6-methoxy-2-naphthyl acetic acid, ibuprofen, probenecid, dansyl-L-asparagine (DNSA), verapamil or disopyramide and the like.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the free fraction of  $^{99m}$ Tc-MAG, in human plasma in the presence of site specific agent.

Fig. 2 shows the free fraction of  $^{99m}$ Tc-MAG, in rat plasma in the presence of site specific agent.

15 Fig. 3 shows the effect of bucolome on blood clearance of  $^{99m}$ Tc-MAG, in rat.

Fig. 4 shows the effect of bucolome on the free fraction of  $^{99m}$ Tc-MAG, in rat blood after administration of bucolome.

20 Fig. 5 shows the effect of bucolome on the accumulation of  $^{99m}$ Tc-MAG, in rat kidney after administration of bucolome.

Fig. 6 shows the effect of bucolome loading on the biodistribution of  $^{99m}$ Tc-MAG, in rat.

25 Fig. 7 shows the renogram of  $^{99m}$ Tc-MAG, in rat.

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## BEST MODE FOR CARRYING OUT OF THE INVENTION

When the second drug having high binding affinity for the same plasma protein, for which the first drug has binding affinity, is administered simultaneously with the first drug, or before or after the administration of the first drug, then competitive displacement will take place at the binding site, thus it can be thought that the first drug may be released in a higher concentration (displacement effect).

Therefore, it can be expected that the higher pharmacological activity of the first drug can be obtained as compared with the case that the first drug is administered singly. On the contrary, when the binding fraction of the first drug to plasma protein increases by the effect of the second drug (reducing effect of the free drug concentration), continuous appearance of pharmacological effect of the first drug can be expected to achieve by keeping the free fraction of the first drug in blood at a lower level for long period.

In the present invention, the first drug with binding affinity for plasma protein may be either one of therapeutic agent or diagnostic agent, as long as it meets the purpose of administration.

Regardless of therapeutic or diagnostic purpose, in case of obtaining the above-mentioned displacement effect, the second drug may be preferably selected from those having competitive binding affinity

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for the same plasma protein as the first drug has; increasing the free fraction of the first drug by the binding inhibition of the first drug with plasma protein; having the affinity for the same binding site 5 of the first drug on plasma protein; and having the higher binding affinity for plasma protein.

On the contrary, in case of obtaining the above-mentioned reducing effect of the free drug concentration, the object is achieved by selecting the 10 second drug from those having effect to increase the binding affinity of the first drug for plasma protein by the second drug bound to the same plasma proteins.

At the present, a report relating to research for clarifying the entity of the reducing effect of the 15 free drug concentration has not been found yet. While, it can be considered that said reducing effect may appear, for example by a mechanism similar to the allosteric effect of an enzyme, and surprisingly, it was found that the binding affinity for the plasma protein 20 could be increased by using the combination of the drugs shown in Example 8 of the present invention.

Regarding dose forms of the drug, in the case that the first drug and second drug are administered simultaneously without necessarily considering any 25 chemical change such as decomposition thereof by mixing together, it is possible to supply a pharmaceutical product prepared by mixing the first drug with the second drug. In such a mixed-type of pharmaceutical

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- preparation, medicinally acceptable ingredients, such as pH-adjusting agents, inorganic salts for adjusting the osmotic pressure, stabilizing agents for stabilizing each one of these ingredients may be added thereto. The  
5 mixed-type of pharmaceutical preparations can be processed into the suitable dose form, for example a liquid form preparation, a lyophilized form preparation and the like, in consideration of the constitutional ingredients, preservation stability thereof, etc.
- 10 Further, the first drug and the second drug may be supplied as a kit form in which they are filled in a container separately. Similar to the mixed-type preparation, medicinally acceptable ingredients, such as stabilizing agents or the like may be added to each one  
15 of these separate type of drugs, and in consideration of administration method, stabilization and the like, these separate type of the drugs can be processed into the suitable form of preparations, such as liquid form preparation, a lyophilized form preparation and the  
20 like.

In case of the kit form mentioned above, the first drug and the second drug can be administered separately, or can be administered simultaneously by mixing together at the time of use. Especially, in the  
25 case of predicting changes of quality of the product, such as decomposition of the ingredients during the storage after mixing the first drug and the second drug, and in the case that these drugs are administered by

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different route, or in the case that these drugs are administered necessarily in different timings, the above-mentioned kit form in which the first drug and the second drugs are filled in separate containers are  
5 useful.

Generally, as the plasma proteins bound to drug, human serum albumin (HSA),  $\alpha_1$ -acidic glycoprotein (AGP),  $\gamma$ -globulin, lipoprotein and the like are exemplified, and many drugs may bind to HSA or AGP.

- 10 In selecting the second drug, for example when the first drug has the property of mainly binding to HSA, it may be preferably selected from an acidic drug having the binding affinity for HSA. When the first drug has the property of binding to AGP, it may be preferably  
15 selected from a basic drug having the binding affinity for AGP. Further, in the case that the first drug has the affinity for plural plasma proteins or has the affinity for different binding sites on the single protein, the use of plural drugs as second drugs may be  
20 effective. Furthermore, in case of selecting the second drug, other properties than the binding affinity with the above-mentioned plasma protein should be considered, such as clinically acceptable appearance of the original pharmacological activity, a broad range of usual dose,  
25 and maintenance of high blood concentration after administration, etc.

Administration timing of the second drug may be either simultaneously with the first drug or before

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or after the administration of the first drug, thus the timing is selected suitably so as to obtain the effect to meet the administration purpose of the first drug.

Administration route of the drugs may be suitably

- 5 selected from either one of intravenous injection,  
intraarterial injection, subcutaneous injection,  
lymphaginal injection or oral administration.

Specifically, HSA has three specific binding sites such as site I, site II and site III on its

- 10 molecule. As the second drug with binding specificity at the site I, the following drugs can be exemplified; bucolome (5-n-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine), cefazolin (7-[1-(H)-tetrazolylacetamido]-3-[2-(5-methyl-1,3,4-

- 15 thiazolyl)thiomethyl]-3-cephem-4-carboxylate), phenylbutazone (1,2-diphenyl-3,5-dioxo-4-n-butyl-pyrazolidine), valproic acid (sodium 2-propylpentanoate), aspirin (2-acetoxybenzoic acid), ceftriaxone
- 20 (disodium (6R,7R)-7-[2-amino-4-thiazoyl]-2-methoxyiminoacetamide)-3-(2,5-dihydro-2-methyl-6-oxide-5-oxo-1,2,4-triazin-3-ylthiomethyl)-8-oxo-5-thia-1-azobicyclo[4.2.0]octo-2-ene-2-carboxylate),

- 25 sulfamethizol (N-(5-methyl-1,3,4-thiadiazol-2-yl)sulfanyl amide), canrenoic acid (17-hydroxy-3-oxo-17 $\alpha$ -pregna-4,6-dien-21-carboxylate), dansyl-L-asparagine, etc. As the second drug with binding specificity at the site II, the following drugs can be exemplified;

ibuprofen (2-(4-isobutylphenyl)propionic acid), nabumetone (4-(6-methoxy-2-naphthyl)-2-butanone (6-methoxy-2-naphthylacetic acid, which is a metabolite of nabumetone, shows binding specificity at the site II) and probenecid (4-(N,N-dipropylsulfamoyl)benzoic acid), etc. Further, etoposide ((5S, 5aR, 8aR, 9S)-9-[(4,6,O-(R)-ethylidene- $\beta$ -D-glucopyranosyl)oxy]-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl-isobenzofuro[5,6-f][1,3]benzodioxol-6(5aH)-one) also has binding specificity for HSA, though the binding site on the HSA has not been assigned. As the second drug with binding specificity for AGP, the following drugs can be exemplified, disopyramide ( $\alpha$ -(2-diisopropylaminoethyl)- $\alpha$ -phenyl-2-pyridineacetamide), verapamil ( $\alpha$ -[3-[[2-(3,4-dimethoxyphenyl)ethyl]-methylamino]propyl]-3,4-dimethoxy- $\alpha$ -(1-methylethyl)benzenecetonitrile) and propranolol (1-isopropylamino-3-(1-naphthoxy)-2-propanol), etc.

As compounds, such as chelating group or recetor ligand, of radio-therapeutic drug for in vivo use or radiodiagnostic drug for in vivo use, both having binding affinity for plasma protein and being labeled with radioactive nuclides, following compounds can be exemplified; mercaptoacetylglycylglycylglycine (MAG) or its derivatives, hexamethylpropyleneaminoxime (HMPAO) or its derivatives, ethylenebis[bis(2-ethoxyethyl)phosphine] (tetrofosmin) or its derivatives, 2,3-dimercaptosuccinic acid (DMSA) or its derivatives,

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ethylene cysteine dimer (ECD) derivatives such as N,N'-ethylene-L-cystein diethylester and the like,  
methoxyisobutylisonitrile (MIBI) derivatives, polyamine  
derivatives such as diethylenetriaminepentaacetic acid  
5 (DTPA) and the like, pyridoxylideneaminate derivatives  
such as pyridoxyleneisoleucine and the like; other  
chelating groups which can form complex with radioactive  
metals such as methylene diphosphonate (MDP),  
hydroxymethylene diphosphonate (HMDP) and the like; and  
10 compounds labeled with radioactive iodine such as  $\beta$ -  
methyl-p-iodophenylpentadecanoic acid (BMIPP), N-  
isopropyl-p-iodoamphetamine (IMP), iodinated hippuric  
acid (OIH), 3-iodobenzylguanidine (MIBG), tropane  
derivatives such as N-(3-fluoropropyl)-2 $\beta$ -carbomethoxy-  
15 3 $\beta$ -(4-iodophenyl)nortropane (FP-CIT), N-methyl-2 $\beta$ -  
carbomethoxy-3 $\beta$ -(4-iodophenyl)nortropane (CIT) and the  
like.

As the radioactive nuclides, following  
nuclides can be exemplified; 11-carbon ( $^{11}\text{C}$ ), 15-oxygen  
20 ( $^{15}\text{O}$ ), 18-fluorine ( $^{18}\text{F}$ ), 32-phosphorus ( $^{32}\text{P}$ ), 59-iron  
( $^{59}\text{Fe}$ ), 67-copper ( $^{67}\text{Cu}$ ), 67-gallium ( $^{67}\text{Ga}$ ), 81m-krypton  
( $^{81\text{m}}\text{Kr}$ ), 81-rubidium ( $^{81}\text{Rb}$ ), 89-strontium ( $^{89}\text{Sr}$ ), 90-  
yttrium ( $^{90}\text{Y}$ ), 99m-technetium ( $^{99\text{m}}\text{Tc}$ ), 111-indium ( $^{111}\text{In}$ ),  
123-iodine ( $^{123}\text{I}$ ), 125-iodine ( $^{125}\text{I}$ ), 131-iodine ( $^{131}\text{I}$ ),  
25 133-xenon ( $^{133}\text{Xe}$ ), 117m-tin ( $^{117\text{m}}\text{Sn}$ ), 153-samarium ( $^{153}\text{Sm}$ ),  
186-rhenium ( $^{186}\text{Re}$ ), 188-rhenium ( $^{188}\text{Re}$ ), 201-thallium  
( $^{201}\text{Tl}$ ), 212-bismuth ( $^{212}\text{Bi}$ ), 213-bismuth ( $^{213}\text{Bi}$ ) and 211-  
astatine ( $^{211}\text{At}$ ). As to the diagnostic purpose, 18-

fluorine ( $^{18}\text{F}$ ),  $^{99\text{m}}\text{Tc}$ -technetium ( $^{99\text{m}}\text{Tc}$ ),  $^{67}\text{gallium}$  ( $^{67}\text{Ga}$ ),  $^{111}\text{indium}$  ( $^{111}\text{In}$ ),  $^{123}\text{iodine}$  ( $^{123}\text{I}$ ),  $^{131}\text{iodine}$  ( $^{131}\text{I}$ ) and the like are frequently used.

5        $^{99\text{m}}\text{Tc}$ -Technetium complex of MAG<sub>3</sub> ( $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub>) is a radiopharmaceutical for *in vivo* use and is widely used for the purpose of diagnosis of renopathy and uropathy, because it possesses accumulation property to the kidney. It is known that about 90% of  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub> bind to plasma protein. For this reason, *in vitro* study was  
10      conducted by using  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub> as the first drug, the serum as the plasma protein, wherein the blood cells and blood coagulation factors are removed, and several pharmaceuticals with the binding affinity for serum proteins as the second drug. As the result, when  
15      bucolome, valproic acid, warfarin or the like was added, then displacement of  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub> occurred either in human serum albumin or in rat serum albumin, thus the free fraction of  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub> in serum albumin increased. In case of bucolome, the free fraction of  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub>,  
20      particularly increased (Table 1). Fig. 5 shows time course of accumulation of  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub> in the rat kidney after administering 20 mg/kg of BucoLome. Fig. 6 shows the biodistributions in rats at 10 minutes after the administration of  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub>. In this case, 10 minutes  
25      before the administration of  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub>, 100 mg/kg of bucolome was administered. These results show that the amount of free  $^{99\text{m}}\text{Tc}$ -MAG<sub>3</sub> was increased by bucolome loading, and rapid clearance from the blood and

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accumulation of  $^{99m}$ Tc-MAG, into the kidney took place.

Regarding  $99m$ -technetium complex of diethyl ester of N,N'-ethylene-L-cystein ( $^{99m}$ Tc-ECD), which is a radiopharmaceutical used for scintigraphy of regional cerebral blood flow, in the *in vitro* experiment by using a human serum, the displacement effect was observed by adding Etoposide (cf. Example 4 and Table 8).

For the purpose to prove the displacement effect on organic compounds, *in vitro* and *in vivo* experiments were conducted by using N-isopropyl-p-iodoamphetamine ( $^{123}$ I-IMP) as one example of organic compounds. In *in vitro* experiments, the displacement effects were observed by adding warfarin or 6-methoxy-2-naphthylacetic acid (6-MNA), both of which have the specificity to HSA, or by adding verapamil which has the binding specificity to AGP (cf. Example 5 and Table 9), thus the displacement effect on organic compounds was observed and proved. Further, in the experiments by using 6-MNA and Verapamil in which those were added separately or added simultaneously, the synergistic effect of the displacement effect was observed, thus it is indicated that the displacement effect can be enhanced by using plural second drugs together (cf. Example 6 and Table 10).

In *in vivo* experiments in rats, as compared with the control group (unloaded with verapamil), the higher concentration of free  $^{123}$ I-IMP in blood was observed in the test group (loaded with verapamil).

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Reflecting the fact, 10 minutes after the administration, the brain uptake of  $^{123}\text{I}$ -IMP in the test group (loaded with verapamil) was about 2 times that in the control group (Example 7). In this in vivo experiments, the test solution containing both of  $^{123}\text{I}$ -IMP and verapamil was prepared in advance (Example 7 (1)) and used it in the experiment. Results of Example 7 indicates that it is possible to regulate the free drug concentration by simultaneous administration of the first and second drugs using their mixture as well as by the separate administration of the first drug and the second drugs and the biodistribution of the first drug could reflect it.

As for an example of the reducing effect of the free drug concentration, decrease in the free fraction (i.e., increase in binding fraction to protein) was observed in in vitro experiment using N-(3-fluoropropyl)- $2\beta$ -carbomethoxy- $3\beta$ -(4-iodophenyl)-nortropane labeled with radioactive iodine ( $\text{I}-125$ ) ( $^{125}\text{I}$ -FP-CIT) together with human serum by adding dansyl-L-asparagine (DNSA) which is specific to the site I on albumin (cf. Example 8 and Table 15).

#### EXAMPLES

The present invention will be explained in more detail by illustrating the following examples, but the invention will not be restricted only to these examples.

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Methods for testing the compounds obtained and the reagents used are as follows.

(1) Ultrafiltration: Filtration was conducted by using an equipment of ULTRACENT-10 which treats for

5 up to 1.5 ml (manufactured by Tosoh Corp.).

(2)  $^{99m}\text{TcO}_4^-$ : Prepared by use of a  $^{99}\text{Mo}/^{99m}\text{Tc}$  generator of MEDITECH (manufactured by NIHON MEDI-PHYSICS CO., LTD.) and used its eluent as in the form of a physiological saline

10 solution.

(3) Reagents: The all reagents used were "Extra-pure reagent grade".

(4) Test animals: The all test animals used were Wister-strain male rats (body weight: 200-250g).

15 Prior to the test, the animals were bred under light-dark cyclic condition in every 12 hours for 1 week, and had free access to food and water.

#### Example 1

##### Examination of displacement effects of the second drugs on $^{99m}\text{Tc-MAG}_3$ binding to plasma protein

Displacement test of  $^{99m}\text{Tc-MAG}_3$  binding to serum albumin was conducted as follows by use of human serum or rat serum and site-specific drugs (second drugs) with binding affinity for the binding site I or site II on albumin. Buclome, valproic acid, warfarin and cefazolin were used as site-specific drugs with

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binding affinity for the site I, and ibuprofen, sodium octanoate and sodium oleate were used as site-specific drugs with binding affinity for the site II.

First, albumin content in a normal human serum was measured previously, and the concentration of the human serum albumin (HSA) was adjusted to 500  $\mu\text{M}$  by phosphate buffer (pH=7.4).

Further, a site-specific drug with the binding affinity for the site I or the site II on HSA was added to the above-mentioned serum solution, as in the form of a methanol solution or an aqueous solution. As the sample solution for control group, only methanol or water was added to the above-mentioned serum solution.

Next, a certain amount of  $^{99\text{m}}\text{Tc}$ -MAG, (about 740 kBq/20  $\mu\text{l}$ ) was added to each of the sample and a certain amount (20-50  $\mu\text{l}$ ) of the sample was taken out as the specimen before ultrafiltration. Each 0.9 mL of the samples was put in a ultrafilter and conducted ultrafiltration under the condition of 1500  $\times$  g, for 10 minutes. Then, 20-50  $\mu\text{l}$  each of the filtrates was taken out as the specimen after ultrafiltration. Radioactivities (cpm) of the specimens before and after ultrafiltration were measured, and the free fraction (%) of  $^{99\text{m}}\text{Tc}$ -MAG, was calculated by the following equation:

$$\text{free fraction of } ^{99\text{m}}\text{Tc}-\text{MAG, } (\%) = [A]/[B]$$

[A]: Radioactivity (cpm) after ultrafiltration,

[B]: Radioactivity (cpm) before ultrafiltration

Similarly, albumin content in normal rat serum

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was previously measured, and the concentration of the rat serum albumin (RSA) was adjusted to 375  $\mu\text{M}$  by phosphate buffer ( $\text{pH}=7.4$ ) so as to conduct the test similar to that of the case of human serum. The results  
5 are shown in Table 1, Fig.1 and Fig.2.

In the case of human serum, the free fraction (%) of  $^{99\text{m}}\text{Tc-MAG}_3$ , in the test samples to which a site-specific drug to site I was added, such as bucolome, valproic acid, warfarin or cefazolin, was significantly  
10 increased as compared with the free fraction (10.2%) of  $^{99\text{m}}\text{Tc-MAG}_3$ , of the control sample.

On the other hand, in other test samples to which a site-specific drug to site II was added, such as ibuprofen, sodium octoate or sodium oleate, increase in  
15 the free fraction was not observed.

Similarly, in test samples of the rat serum to which a site-specific drug to site I was added, increase in the free fraction (%) of  $^{99\text{m}}\text{Tc-MAG}_3$ , was observed.

As can be seen from the above results, it is  
20 clearly indicated that the free fraction of  $^{99\text{m}}\text{Tc-MAG}_3$  in the blood can be increased by adding a site-specific drug to site I. Although, warfarin, octanoic acid and oleic acid might be considered clinically unsuitable for  
25 the purpose of this invention, they were used for the confirmation of the effects of site-specific drugs to the binding site.

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Table 1. Displacement effects of the second drugs on  $^{99m}\text{Tc}$ -MAG<sub>3</sub> binding to plasma protein

Site-specific drug (second drug) concentration	Human serum albumin (HAS) $^{99m}\text{Tc}$ -MAG <sub>3</sub> free fraction (%)		Rat serum albumin (RSA) $^{99m}\text{Tc}$ -MAG <sub>3</sub> free fraction (%)	
	200 $\mu\text{M}$	400 $\mu\text{M}$	200 $\mu\text{M}$	400 $\mu\text{M}$
Control	10.20%	10.20%	24.75%	24.75%
bucolome	12.23%	13.74%	32.76%	43.85%
valproic acid	11.98%	13.02%	28.48%	29.30%
warfarin	11.50%	13.57%	33.57%	43.28%
cefazolin	11.13%	14.76%	28.58%	33.52%
ibuprofen	10.18%	10.53%	28.48%	33.04%
octanoic acid	9.60%	9.86%	—	—
oleic acid	8.74%	9.44%	—	—

## Example 2

Biodistribution of  $^{99m}$ Tc-MAG<sub>3</sub> in rat loaded with bucolome

Effect of second drug on biodistribution of  $^{99m}$ Tc-MAG<sub>3</sub> in rat was examined using the control group and the test group with bucolome loading.  $^{99m}$ Tc-MAG<sub>3</sub> (740 kBq/100  $\mu$ l) was administered to the tail vein of Wister-strain rat. The rats were decapitated at 2, 5, 10 and 15 minutes after administration of  $^{99m}$ Tc-MAG<sub>3</sub>, then the blood and the organs of interest were excised.

After measured the weight of these organs, the radioactivities were determined. After decay correction of the radioactivity, the accumulation ratios (% dose/organ and % dose/g of the tissue) were determined.

As for the rat of test group loaded with bucolome, 5 minutes before the administration of  $^{99m}$ Tc-MAG<sub>3</sub>, 20 mg/kg of body weight or 100 mg/kg of body weight of bucolome was administered to the tail vein.

The results are shown in Table 2 and Table 3 (control group), Table 4 and Table 5 (test group, loaded with 20 mg/kg of bucolome) and Table 6 (test group, loaded with 100 mg/kg of bucolome).

In the control group and test group with bucolome loading of 20 mg/kg of body weight, wherein the dose and other conditions were the same as mentioned above, except the decapitation time was prescribed at 2, 5 and 10 minutes, administrations and decapitations of rats were conducted so that 3-5 ml of blood per one rat were collected. Serum was separated using a sample

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tube, after that the free fraction was determined by the procedures as described in Example 1. The time course of the free fraction of  $^{99m}\text{Tc}$ -MAG, in vivo are shown in Fig. 4.

5 From the results shown above, it has become apparent that blood clearance was accelerated in the test group with bucolome loading (Fig. 3), and the free fraction of  $^{99m}\text{Tc}$ -MAG, in vivo in test group was remarkably increased (Fig. 4).

10 In the control group, accumulation of  $^{99m}\text{Tc}$ -MAG, in the kidney (% dose/organ) increased from 2 minutes to 5 minutes after the administration, then gradually decreased and disappeared. While, in test group with bucolome loading, accumulation of  $^{99m}\text{Tc}$ -MAG,  
15 in the kidney rapidly increased to the maximum value just after the administration (in 2 minutes), then decreased and disappeared quickly as compared with that of the control group (Fig. 5).

20 Biodistribution of  $^{99m}\text{Tc}$ -MAG, (% dose/g tissue) in rat 10 minutes after the administration is shown in Fig. 6. As can be seen from Fig. 6, in the test group with bucolome loading,  $^{99m}\text{Tc}$ -MAG, was rapidly cleared from the kidney which is the target organ of  $^{99m}\text{Tc}$ -MAG, thus radioactivity was quickly cleared as  
25 compared with that of the control group. Clearance from the blood and other organs were also rapid.

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Table 2. Biodistribution of  $^{99m}\text{Tc}$ -MAG<sub>3</sub>  
in rats (Control group: % dose/organ)

Organs	2 minutes	5 minutes	10 minutes	15 minutes
Spleen	0.110±0.024	0.064±0.001	0.025±0.006	0.014±0.001
Pancreas	0.137±0.026	0.079±0.013	0.084±0.050	0.030±0.001
Stomach	0.276±0.011	0.169±0.003	0.119±0.043	0.178±0.009
Liver	5.196±0.387	5.187±2.759	1.671±0.099	0.973±0.266
Kidney	23.882±4.669	31.324±4.979	29.198±3.729	15.864±3.960
Heart	0.262±0.039	0.184±0.046	0.079±0.028	0.034±0.004
Lung	0.635±0.116	0.594±0.106	0.275±0.042	0.129±0.084
Urine	0.236±0.119	1.309±0.941	16.872±4.042	38.419±2.150

Table 3. Biodistribution of  $^{99m}$ Tc-MAG,  
in rats (Control group: % dose/g tissue)

Organs	2 minutes	5 minutes	10 minutes	15 minutes
Blood	1.482±0.137	0.968±0.163	0.387±0.018	0.160±0.022
Spleen	0.171±0.031	0.104±0.014	0.044±0.008	0.026±0.006
Pancreas	0.233±0.029	0.150±0.001	0.096±0.027	0.048±0.007
Stomach	0.176±0.011	0.031±0.012	0.015±0.010	0.110±0.050
Liver	0.579±0.081	0.523±0.268	0.145±0.009	0.100±0.024
Kidney	13.039±3.194	16.721±0.992	15.526±2.763	8.282±1.222
Heart	0.448±0.056	0.290±0.044	0.128±0.031	0.057±0.010
Lung	0.621±0.100	0.470±0.064	0.213±0.008	0.112±0.057

## TOTAL SITUATION

Table 4. Biodistribution of  $^{99m}\text{Tc}$ -MAG<sub>3</sub> in rats  
 ("Test group with 20 mg/kg bucolome  
 loading: % dose/organ)

Organs	2 minutes	5 minutes	10 minutes	15 minutes
Spleen	0.103±0.001	0.048±0.006	0.018±0.009	0.011±0.003
Pancreas	0.239±0.072	0.139±0.030	0.060±0.023	0.075±0.053
Stomach	0.289±0.057	0.153±0.023	0.111±0.032	0.104±0.040
Liver	7.289±0.333	3.140±0.745	1.217±0.471	0.806±0.187
Kidney	26.404±2.243	22.952±9.437	17.118±8.295	9.544±3.655
Heart	0.210±0.034	0.114±0.019	0.037±0.012	0.029±0.014
Lung	0.742±0.044	0.456±0.137	0.148±0.079	0.085±0.025
Urine	0.802±0.709	2.692±2.721	14.792±4.307	23.969±18.025

Table 5. Biodistribution of  $^{99m}\text{Tc}$ -MAG<sub>3</sub> in rats  
 (Test group with 20 mg/kg bucolome  
 loading: % dose/g tissue)

Organs	2 minutes	5 minutes	10 minutes	15 minutes
Blood	1.050±0.057	0.544±0.043	0.188±0.076	0.152±0.088
Spleen	0.153±0.018	0.083±0.005	0.026±0.011	0.018±0.006
Pancreas	0.314±0.013	0.145±0.017	0.062±0.021	0.088±0.052
Stomach	0.145±0.121	0.033±0.017	0.033±0.020	0.032±0.012
Liver	0.853±0.135	0.280±0.017	0.117±0.035	0.088±0.028
Kidney	13.069±0.379	11.050±4.260	8.558±3.867	4.809±1.823
Heart	0.329±0.034	0.172±0.021	0.057±0.017	0.045±0.021
Lung	0.613±0.013	0.373±0.073	0.120±0.049	0.081±0.020

Table 6. Biodistribution of  $^{99m}\text{Tc}$ -MAG<sub>3</sub> in rats  
 10 minutes after the administration  
 (Test group with 100 mg/kg bucolome  
 loading: % dose/g tissue)

Organs	Control group	Test group with bucolome loading
Blood	0.317±0.073	0.047±0.044
Brain	0.010±0.001	0.001±0.001
Spleen	0.052±0.008	0.009±0.008
Pancreas	0.046±0.000	0.006±0.007
Stomach	0.024±0.024	0.040±0.036
Liver	0.151±0.001	0.033±0.026
Kidney	6.191±0.187	0.651±0.324
Heart	0.101±0.016	0.014±0.010
Lung	0.195±0.030	0.043±0.037

5      Example 3

Examination of displacement effect on  $^{99m}\text{Tc}$ -MAG<sub>3</sub> by means  
 of renography in rats

By using Wistar-strain rats (body weight:  
 400g), displacement effect of bucolome on  $^{99m}\text{Tc}$ -MAG<sub>3</sub> was  
 10     examined by means of renography in rats. Prism 3000  
 (picker) was used as an apparatus.

A catheter was inserted into the femoral vein  
 of the rat, then  $^{99m}\text{Tc}$ -MAG<sub>3</sub> (11.1 MBq) was injected

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through the catheter and the control renogram was obtained. The dynamic imaging was conducted 10 seconds/scan for 20 minutes. About 2 hours after, confirming the urination and decrease in background 5 radioactivity, then bucolome was loaded to the same rat. Bucolome was dissolved in ethanol and adjusted as in the dose of 20 mg/kg, then intravenously injected by using a microinjector taken in 10 minutes. About 5 minutes after finish of the intravenous injection of bucolome, 10  $^{99m}\text{Tc}$ -MAG<sub>3</sub> was intravenously injected through the catheter and the renogram was taken similarly by 10 seconds/scan for 20 minutes. Fig. 7 shows the renogram (time-radioactivity curves in the kidney) used for functional analysis of the kidney. As can be seen from 15 the Fig. 7, in the control group, the radioactivity curve went up gradually at the initial stage after the administration, and the peak time was 240 seconds. On the other hand, in the test group with bucolome loading, the radioactivity curve rose quickly, and the peak time 20 was 120 seconds which was a half length of that of the control group. Renal function is usually analyzed by determination of the peak time in this renogram and a slope of the straight line in a linear regression. By inhibiting the binding ability of  $^{99m}\text{Tc}$ -MAG<sub>3</sub> to plasma 25 protein, the renogram was obtained as close to an ideal and was approximated to a simple curve. Thus, the functional analysis of the kidney can be easily carried out, and the time for functional analysis can be

shortened by shortening the peak time.

Table 7. Analytical results of renogram  
of  $^{99m}\text{Tc}$ -MAG<sub>3</sub> in rats

	Peak time (Second)	Slope (Count/second)
<b>Rat 1</b>		
Control group	240	1.166
Test group (with bucolome loading)	110	2.208
<b>Rat 2</b>		
Control group	170	0.941
Test group (with bucolome loading)	120	2.000

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## Example 4

Examination of displacement effects of the second drugs  
on  $^{99m}$ Tc-EDC binding to plasma protein

Displacement experiment of  $^{99m}$ Tc-ECD binding to a serum albumin was conducted by procedures similar to those shown in Example 1 using human serum; and bucolome, valproic acid, warfarin and cefazolin with binding specificity to the site I on albumin; ibuprofen and sodium octanoate with binding specificity to the site II on alubumin; and etoposide with binding specificity to HAS on which binding site is not identified. The results are shown in Table 8.

As compared with the free fraction (26.03%) of  $^{99m}$ Tc-ECD in human serum shown in control group, the free fraction of  $^{99m}$ Tc-ECD in human serum were remarkably increased in test group by etoposide, both at the concentration of 200  $\mu$ M and 400  $\mu$ M. Similarly, the free fraction of  $^{99m}$ Tc-ECD in human serum were also incresed by bucolome, valproic acid and warfarin, but were not remarkably shown as compared with that of etoposide. On the contrary, the free fraction of  $^{99m}$ Tc-ECD in human serum were not clearly increased by ibuprofen and sodium octanoate which have the specificity to the site II on albumine.

Table 8 Displacement of  $^{99m}$ Tc-ECD binding  
to plasma protein

Site-specific drug (second drug)	free fraction of $^{99m}$ Tc-ECD (%)	
Concentration	200 $\mu$ M	400 $\mu$ M
Control		26.03%
Bucolome	28.62%	30.25%
Valproic acid	28.36%	30.25%
Warfarin	31.00%	31.37%
Cefazolin	25.92%	27.40%
Etoposide	33.26%	37.38%
Ibuprofen	23.09%	24.09%
Octanoic acid	28.22%	29.64%

Example 5

5     Examination of displacement effects of the second drugs  
on  $^{123}$ I-IMP binding to plasma protein

Displacement experiment of  $^{123}$ I-IMP binding to a serum albumin was conducted by procedures similar to those shown in Example 1 using human serum; and as the 10 second drug, bucolome and warfarin with binding specificity to the binding site I on albumin; ibuprofen, sodium octanoate, 6-methoxy-2-naphthylacetic acid (6-MNA) with binding specificity to the binding site II on alubumin; and verapamil which has the specificity to  $\alpha_1$ -acid glycoprotein (AGP). Concentration of the second 15 drug (e.g., bucolome) was 400  $\mu$ M and added amount of

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$^{123}\text{I}$ -IMP was about 220 kBq/20  $\mu\text{L}$ . The results are shown in Table 9.

- As compared with free fraction (29.29%) of  $^{123}\text{I}$ -IMP in human serum shown in control group, the free fraction of  $^{123}\text{I}$ -IMP in human serum in test group was remarkably increased by adding verapamil with binding specificity to AGP. Further, the free fraction of  $^{123}\text{I}$ -IMP binding in human serum in test group was also increased by warfarin and 6-MNA mainly bound to albumin.
- In view of these facts, it is suggested that  $^{123}\text{I}$ -IMP binds to the binding site on both albumin and AGP, and it is clearly understood that the free fraction of  $^{123}\text{I}$ -IMP can be increased by a drug having the specificity to each binding sites of these proteins.

Table 9. Displacement of  $^{123}\text{I}$ -IMP binding to  
human plasma protein (Concentration of  
the site-specific drug was 400  $\mu\text{M}$ )

Site-specific drug (second drug)	free of fraction of $^{123}\text{I}$ -IMP (%)
Control	29.29%
bucolome	30.26%
warfarin	34.69%
ibuprofen	28.43%
octanoic acid	28.74%
6-MNA	32.70%
verapamil	38.34%

Example 6

Examination of displacement effects of the second drugs  
on  $^{123}\text{I}$ -IMP binding to plasma protein: synergistic effect

Displacement experiment of  $^{123}\text{I}$ -IMP binding to  
5 serum albumin was conducted by procedures similar to  
those shown in Example 5 using human serum and as the  
second drugs, 6-MNA having the specificity to the  
binding site II on albumin and verapamil having the  
specificity to the binding site on AGP. Concentration  
10 of the second drugs were 400  $\mu\text{M}$  and the added amount of  
 $^{123}\text{I}$ -IMP was about 220 kBq/20  $\mu\text{L}$ .

The tests were conducted in one group by using  
6-MNA or verapamil independently, and in another group  
by using 6-MNA and verapamil simultaneously to study  
15 synergistic effect. In both groups, concentrations of

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the second drugs were 400  $\mu\text{M}$ . The results are shown in Table 10.

In case of using 6-MNA and verapamil simultaneously, the free fraction of  $^{123}\text{I}$ -IMP was over 5 than the sum of the corresponding values obtained by single use of 6-MNA or verapamil, respectively. In view of the above facts, synergistic effect can be expected by using the plural second drugs.

Table 10. Displacement of  $^{123}\text{I}$ -IMP binding to human plasma protein: synergistic effect

Site-specific drug (second drug)	free fraction of $^{123}\text{I}$ -IMP
Control	26.52%
6-MNA	30.00%
verapamil	33.87%
6-MNA + verapamil	39.26%

#### Example 7

##### Biodistribution of $^{123}\text{I}$ -IMP in rat with verapamil loading

(1) Preparation of  $^{123}\text{I}$ -IMP·verapamil mixed solution

35 Milligrams of verapamil bulk drug powder 15 was dissolved in 2 ml of Vasolan injection (verapamil 5 mg/2 ml, manufactured by Eisai Co., Ltd.), then 34  $\mu\text{L}$  of  $^{123}\text{I}$ -IMP injection (111 MBq/ml, manufactured by NIHON MEDI-PHYSICS CO., LTD.) was added thereto and mixed

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throughly.

(2) Biodistribution of  $^{123}\text{I}$ -IMP in rats

Control group:  $^{123}\text{I}$ -IMP Injection solution (185 kBq/300  $\mu\text{L}$ ) being diluted with physiological saline was administered via the caudal vein of rats of control group. The rats were decapitated at 2, 5, 10, 30 and 60 minutes after administration. Then the blood was sampled and the organs of interest were excised. After measured the weight of these specimens, the radioactivities of the blood and organs were measured. After half-life of the radioactivities were corrected, the accumulation rate (% dose/g of the tissue) was obtained.

Test group: 100  $\mu\text{L}$  of  $^{123}\text{I}$ -IMP-verapamil mixed solution was administered via the caudal vein of rats of the test group (about 10 mg/kg loaded as verapamil), then the rats were treated similarly to those of control group. The results of biodistribution of  $^{123}\text{I}$ -IMP are shown in Table 11 (control group), Table 12 (test group with verapamil loading), and Table 13 (comparison of both control and test groups of 10 minutes after the administration).

(3) Examination of displacement effect on  $^{123}\text{I}$ -IMP binding to plasma protein in rat

Under the same conditions as mentioned above concerning the constitution of control and testing

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group, timings of decapitation, and dose of drugs, administrations of the drugs and decapitations of rats were conducted, and 3-5 ml of blood per one rat was sampled. Serum was separated using a sample tube, after 5 that the free fraction of  $^{123}\text{I}$ -IMP was determined by the procedures as described in Example 1. The free fraction of  $^{123}\text{I}$ -IMP in the blood sample of rat obtained at each decapitation timing are shown in Table 14.

As shown in Table 14, it is clearly indicated 10 that the free fraction of  $^{123}\text{I}$ -IMP in the blood sample of rat were increased by the loading with the verapamil. As shown in Table 11 through Table 13, corresponding to increasing in the free fraction of  $^{123}\text{I}$ -IMP in the blood due to the loading with verapamil, the uptake of 15  $^{123}\text{I}$ -IMP into the brain which is the target organ of  $^{123}\text{I}$ -IMP was rapidly increased after administration of  $^{123}\text{I}$ -IMP-verapamil mixed solution in the test group, thus the brain uptake of  $^{123}\text{I}$ -IMP in the test group after the administration was increased about 2 times higher than 20 that of shown in control group. These facts indicate that, even if a mixed drug of the first drug and the second drug is administered (simultaneous administration of the first drug and the second drug), the free fraction of  $^{123}\text{I}$ -IMP can be regulated by the second drug, 25 and the biodistribution of the first drug could reflect it.

Table 11. Biodistribution of  $^{123}\text{I}$ -IMP in rats  
 (Control group: % Dose/g Tissue)

Tissues	2 Minutes	5 Minutes	10 Minutes	30 Minutes	60 Minutes
Blood	0.198±0.052	0.133±0.005	0.116±0.011	0.136±0.028	0.181±0.006
Brain	1.800±0.418	1.476±0.225	1.006±0.379	1.346±0.345	1.511±0.011
Pancreas	1.503±0.353	1.923±0.445	1.721±0.217	2.032±0.505	1.957±0.345
Spleen	0.880±0.216	0.999±0.355	1.008±0.074	1.356±0.277	1.290±0.138
Stomach	0.302±0.065	0.500±0.078	0.407±0.230	0.885±0.366	1.245±0.343
Liver	0.506±0.109	0.699±0.061	0.711±0.143	1.192±0.536	1.442±0.164
Kidney	3.406±0.905	2.285±0.256	1.303±0.190	1.359±0.222	1.585±0.132
Heart	1.949±0.293	1.014±0.070	0.631±0.111	0.524±0.037	0.540±0.026
Lung	11.236±0.780	9.000±0.600	6.279±1.026	5.209±1.446	5.186±0.616

Table 12. Biodistribution of  $^{123}\text{I}$ -IMP in rats  
 [Test group (Loaded with verapamil): % Dose/g Tissue]

Tissues	2 Minutes	5 Minutes	10 Minutes	30 Minutes	60 Minutes
Blood	0.238±0.083	0.228±0.012	0.139±0.003	0.098±0.044	0.110±0.002
Brain	1.584±0.425	1.916±0.131	2.145±0.410	1.529±0.811	1.449±0.281
Pancreas	1.268±0.375	1.659±0.496	1.911±0.685	1.877±0.886	1.478±0.161
Spleen	0.052±0.025	0.063±0.250	0.213±0.118	0.886±0.319	1.193±0.129
Stomach	0.234±0.111	0.164±0.078	0.377±0.013	0.782±0.621	1.058±0.126
Liver	0.287±0.156	0.350±0.130	0.688±0.237	1.185±0.751	1.639±0.051
Kidney	1.424±0.313	1.278±0.381	1.766±0.678	1.231±0.632	1.242±0.146
Heart	3.769±0.911	2.260±0.680	1.247±0.209	0.471±0.209	0.456±0.039
Lung	9.234±1.748	8.377±0.563	6.947±1.486	3.890±2.223	4.133±0.079

Table 13. Biodistribution of  $^{123}\text{I}$ -IMP in rats  
 10 minutes after the administration  
 (% Dose/g Tissue)

Tissue	Control group	Test group with verapamil loading
Blood	$0.116 \pm 0.011$	$0.139 \pm 0.003$
Brain	$1.006 \pm 0.379$	$2.145 \pm 0.410$
Pancreas	$1.721 \pm 0.217$	$1.911 \pm 0.685$
Spleen	$1.008 \pm 0.074$	$0.213 \pm 0.118$
Stomach	$0.407 \pm 0.230$	$0.377 \pm 0.013$
Liver	$0.711 \pm 0.143$	$0.688 \pm 0.237$
Kidney	$1.303 \pm 0.190$	$1.766 \pm 0.678$
Heart	$0.631 \pm 0.111$	$1.247 \pm 0.209$
Lung	$6.279 \pm 1.026$	$6.947 \pm 1.486$

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Table 14. Free fraction (%) of  $^{123}\text{I}$ -IMP in the blood of rats

	2 Minutes	5 Minutes	10 Minutes	30 Minutes	60 Minutes
Control group	56.75±9.21	50.70±10.37	45.91±3.12	27.29±4.85	16.77±4.11
Test group with verapamil loading	52.40±6.00	56.52±4.38	66.86±6.34	38.03±6.69	31.86±8.23

## Example 8

Examination of regulating the free fraction of  
 $^{125}\text{I}$ -FP-CIT

Experiment was conducted by procedures  
5 similar to those shown in Example 5 using human serum  
and as the second drug, bucolome, phenylbutazone,  
warfarin and dansyl-L-asparagine (DNSA) with binding  
specificity to the binding site I on albumin, and  
ibuprofen, 6-methoxy-2-naphthylacetic acid (6-MNA) with  
10 binding specificity to the binding site II on albumin.  
Concentration of the second drug (e.g., bucolome and  
the like) were 400  $\mu\text{M}$  and added amount of  $^{123}\text{I}$ -FP-CIT  
was about 74 kBq/20  $\mu\text{L}$ . The result are shown in Table  
15.

15 As compared with the free fraction (17.26%)  
of  $^{123}\text{I}$ -FP-CIT in human serum shown in control group, the  
free fraction of  $^{123}\text{I}$ -FP-CIT in test group was remarkably  
decreased by DNSA. Furthermore, the free fraction of  
 $^{123}\text{I}$ -FP-CIT in test group was also decreased by  
20 phenylbutazone and ibuprofen. In view of these facts,  
it is clearly understood that the free fraction of the  
first drug can be decreased by the second drug having  
the binding affinity for the plasma proteins.

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Table 15. Free fraction of  $^{123}\text{I}$ -FP-CIT in human serum (concentration of the site-specific drug was 400  $\mu\text{M}$ )

Site-specific drug (second drug)	free fraction (%) of $^{123}\text{I}$ -FP-CIT
Control	17.26%
bucolome	18.40%
phenylbutazone	14.92%
warfarin	17.88%
DNSA	12.80%
ibuprofe	15.92%
6-MNA	18.10%

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CLAIMS as filed in the Amendment under PCT Article 34

1. (Amended) A pharmaceutical preparation with binding affinity for plasma protein which comprises a single or plural second drug, characterized in that the 5 second drug has binding affinity for the same plasma protein for which a first drug has binding affinity and the pharmaceutical preparation is administered simultaneously with the first drug or before or after the administration of the first drug to thereby regulate the binding of the first drug to the plasma 10 protein.
  2. (Amended) The pharmaceutical preparation according to Claim 1, wherein the second drug has binding affinity to the same binding sites on the 15 plasma protein to which the first drug has binding affinity.
  3. (Amended) The pharmaceutical preparation according to Claim 1 or 2, wherein the first drug is a radiodiagnostic drug for in vivo use or the 20 radiotherapeutic drug for in vivo use.
  4. (Amended) The pharmaceutical preparation according to Claim 3, wherein the radiodiagnostic drug for in vivo use or the radiotherapeutic drug for in vivo use is radiolabeled with one nuclide selected from 25 the group consisting of 11-carbon (<sup>11</sup>C), 15-oxygen (<sup>15</sup>O), 18-fluorine, (<sup>18</sup>F), 32-phosphorus (<sup>32</sup>P), 59-iron (<sup>59</sup>Fe), 67-copper (<sup>67</sup>Cu), 67-gallium (<sup>67</sup>Ga), 81m-krypton (<sup>81m</sup>Kr), 81-rubidium (<sup>81</sup>Rb), 89-strontium (<sup>89</sup>Sr), 90-yttrium (<sup>90</sup>Y),

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99m-technetium ( $^{99m}\text{Tc}$ ), 111-indium ( $^{111}\text{In}$ ), 123-iodine ( $^{123}\text{I}$ ), 125-iodine ( $^{125}\text{I}$ ), 131-iodine ( $^{131}\text{I}$ ), 133-xenon ( $^{133}\text{Xe}$ ), 117m-tin ( $^{117m}\text{Sn}$ ), 153-samarium ( $^{153}\text{Sm}$ ), 186-rhenium ( $^{186}\text{Re}$ ), 188-rhenium ( $^{188}\text{Re}$ ), 201-thallium ( $^{201}\text{Tl}$ ),  
5 212-bismuth ( $^{212}\text{Bi}$ ), 213-bismuth ( $^{213}\text{Bi}$ ) and 211-astatine ( $^{211}\text{At}$ ).

5. (Amended) The pharmaceutical preparation according to Claim 3, wherein the first drug has one group labeled with nuclide and the group is selected  
10 from the group consisting of bisaminothiol or its derivatives, monoaminomonooamidobisthiol or its derivatives, bisamidobisthiol or its derivatives, mercaptoacetyl-glycylglycylglycine or its derivatives, hexamethylpropyleneamineoxime or its derivatives,  
15 ethylenebis[bis(2-ethoxyethyl)phosphine] (tetrofosmin) or its derivatives, 2,3-dimercaptosuccinic acid or its derivatives, ethylenecysteine dimer derivatives, methoxyisobutylisonitrile derivatives, polyamine derivatives, pyridoxylydeneaminate derivatives,  
20 methylene diphosphonate, hydroxymethylene diphosphonate derivative,  $\beta$ -methyl- $\omega$ -phenylpentadecanoic acid or its derivatives, N-isopropylamphetamine, hippuric acid and benzylguanidine and tropane derivatives.

6. (Amended) The pharmaceutical preparation according to any one of claims 1 to 3, wherein the single or plural second drug is selected from the group consisting of bucolome, cefazolin, etoposide, phenylbutazone, aspirine, salicylic acid, cefatriaxone,

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sulfamethizole, valproic acid, nabumetone, 6-methoxy-6-naphthyl acetic acid, ibuprofen, probenecid, dansyl-L-asparagine, verapamil and disopyramide.

7. A pharmaceutical preparation characterized by  
5 regulating binding affinity of a first drug for plasma protein, which comprises a first drug with binding affinity for plasma protein and a single or plural second drug with binding affinity for the same plasma protein, for which the first drug has binding affinity.

10 8. The pharmaceutical preparation according to  
Claim 7, wherein each of the first drug and the second drug is separately filled in a container, and prepared as kit form for supply.

9. The pharmaceutical preparation according to  
15 Claim 7 or 8, wherein the second drug has binding affinity to the same binding sites on the plasma protein, to which the first drug has binding affinity.

10. The pharmaceutical preparation according to  
any one of Claims 7 to 9, wherein the first drug is  
20 a radiodiagnostic drug for in vivo use or a  
radiotherapeutic drug for in vivo use.

11. The pharmaceutical preparation according to  
Claim 10, wherein the radiodiagnostic drug for in vivo use or the radiotherapeutic drug for in vivo use is  
25 radiolabeled with one nuclide selected from the group consisting of 11-carbon (<sup>11</sup>C), 15-oxygen (<sup>15</sup>O), 18-fluorine (<sup>18</sup>F), 32-phosphorus (<sup>32</sup>P), 59-iron (<sup>59</sup>Fe), 67-copper (<sup>67</sup>Cu), 67-gallium (<sup>67</sup>Ga), 81m-krypton (<sup>81m</sup>Kr), 81-

rubidium ( $^{85}\text{Rb}$ ), 89-strontium ( $^{89}\text{Sr}$ ), 90-yttrium ( $^{90}\text{Y}$ ),  
99m-technetium ( $^{99\text{m}}\text{Tc}$ ), 111-indium ( $^{111}\text{In}$ ), 123-iodine  
( $^{123}\text{I}$ ), 125-iodine ( $^{125}\text{I}$ ), 131-iodine ( $^{131}\text{I}$ ), 133-xenon  
( $^{133}\text{Xe}$ ), 117m-tin ( $^{117\text{m}}\text{Sn}$ ), 153-samarium ( $^{153}\text{Sm}$ ), 186-  
5 rhenium ( $^{186}\text{Re}$ ), 188-rhenium ( $^{188}\text{Re}$ ), 201-thallium ( $^{201}\text{Tl}$ ),  
212-bismuth ( $^{212}\text{Bi}$ ), 213-bismuth ( $^{213}\text{Bi}$ ) and 211-astatine  
( $^{211}\text{At}$ ).

12. The pharmaceutical preparation according to  
Claim 10, wherein the first drug has one group labeled  
10 with nuclide and the group is selected from the group  
consisting of bisaminothiol or its derivatives,  
monoaminomonooamidobisthiol or its derivatives,  
bisamidobisthiol or its derivatives, mercapto-  
acetylglycylglycylglycine or its derivatives,  
15 hexamethylpropyleneamineoxime or its derivatives,  
ethylenebis[bis(2-ethoxyethyl)phosphine] (tetrofosmin)  
or its derivatives, 2,3-dimercaptosuccinic acid or its  
derivatives, ethylenecysteine dimer derivatives  
methoxyisobutylisonitrile derivatives, polyamine  
20 derivatives, pyridoxylydeneamine derivatives,  
methylene diphosphonate, hydroxymethylene diphosphonate  
derivatives,  $\beta$ -methyl- $\omega$ -phenylpentadecanoic acid or  
its derivatives, N-isopropylamphetamine, hippuric acid,  
benzylguanidine and tropane derivatives.
- 25 13. The pharmaceutical preparation according to  
any one of Claims 7 to 10, wherein the single or plural  
second drugs is selected from the group consisting of  
bucolome, cefazolin, etoposide, phenylbutazone,

aspirine, salicylic acid, ceftriaxone, sulfamethizole,  
valproic acid, nabumetone, 6-methoxy-2-naphthylacetic  
acid, ibuprofen, probenecid, dansyl-L-asparagine,  
verapamil and disopyramide.

## ABSTRACT

A method of the administration of drugs with binding affinity for plasma protein and drugs regulating the effective ingredient dose of drugs with 5 binding affinity for plasma protein; and a preparation whereby the effective ingredient dose of drugs with binding affinity for plasma protein is regulated. The above administration method is characterized in that, in the administration of a first drug having binding 10 affinity for plasma protein, a second drug having binding affinity for the same plasma protein is administered simultaneously with the first drug or before or after the administration of the first drug to thereby regulate the binding of the first drug to the 15 plasma protein.

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FIG.1

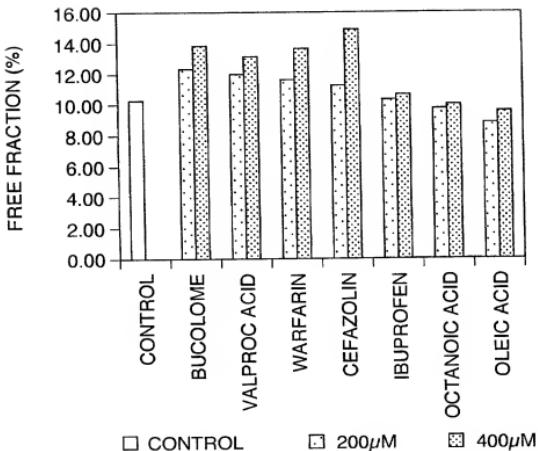
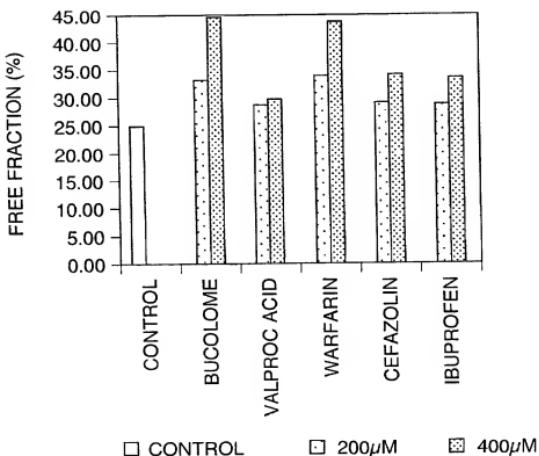


FIG.2



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FIG.3

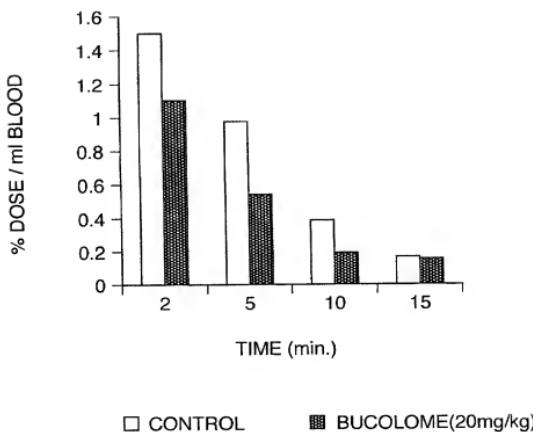
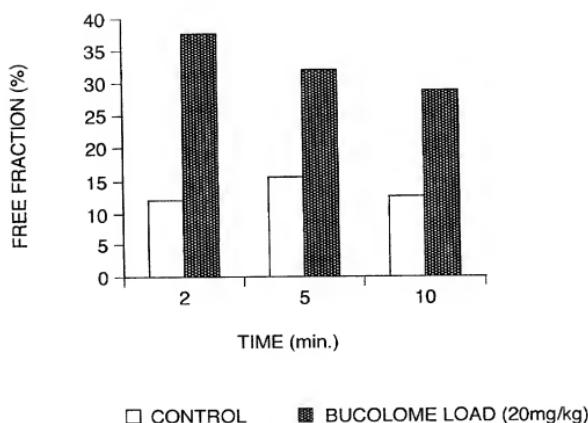


FIG.4



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FIG.5

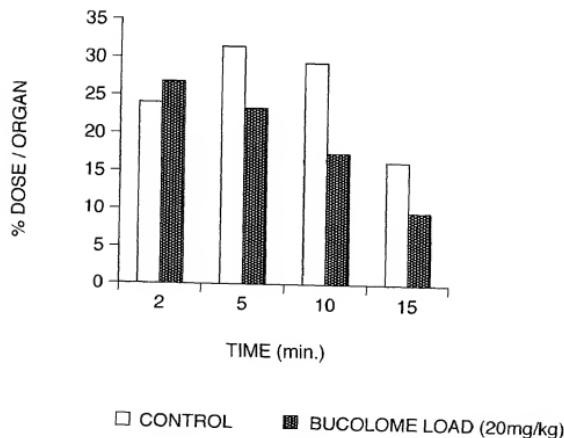
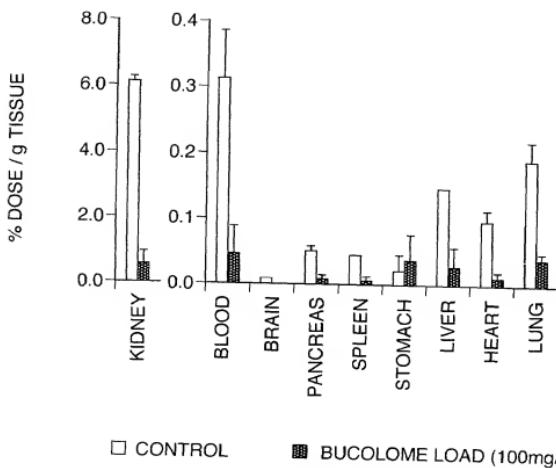


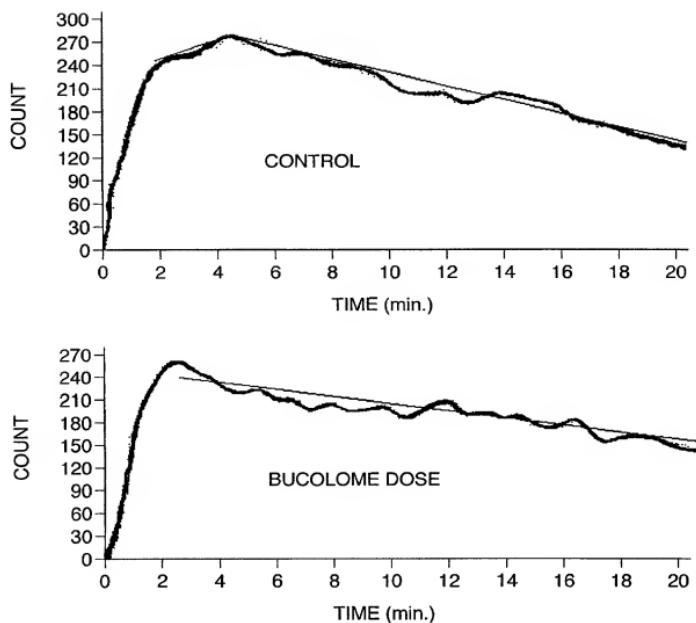
FIG.6



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FIG.7



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DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name: that I verily believe I am the original, first and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought in the application entitled:

**"METHOD OF THE ADMINISTRATION OF DRUGS WITH BINDING AFFINITY FOR PLASMA PROTEIN AND PREPARATION TO BE USED IN THE METHOD"**

which application is:

the attached application  
(for original application)

application Serial No. PCT/JP00/04039  
filed June 21, 2000, and amended on  
January 12, 2001  
(for declaration not accompanying application)

that I have reviewed and understand the contents of the specification of the above-identified application, including the claims, as amended by any amendment referred to above; that I acknowledge my duty to disclose information of which I am aware which is material to the patentability of this application under 37 C.F.R. 1.56, that I hereby claim foreign priority benefits under Title 35, United States Code §119, §172 or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified on said list any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application on which priority is claimed:

	Application Number	Country	Filing Date	Priority Claimed (yes or no)
<input type="checkbox"/>	11-173514	Japan	June, 21, 1999	yes

I hereby claim the benefit of Title 35, United States Code §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in a listed prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge my duty to disclose any information material to the patentability of this application under 37 C.F.R. 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

	Application Serial No.	Filing Date	Status (patented, pending, abandoned)
<input type="checkbox"/>			

I hereby appoint John H. Mion, Reg. No. 18,879; Donald E. Zinn, Reg. No. 19,046; Thomas J. Macpeak, Reg. No. 19,292; Robert H. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Robert G. McMorrow, Reg. No. 19,093; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562; Brian W. Hannon, Reg. No. 32,778 and Abraham J. Rosner, Reg. No. 33,276, my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to SUGHRUE, MION, ZINN, MACPEAK & SEAS, 2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037-3202.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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